

# Research Paper: Studying Renal Artery Bifurcation Structure in Male Dogs

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## **ABSTRACT**

**Introduction:** Arteries are made up three layers; tunica intima, tunica media, and tunica adventitia. However, in some part of the artery this structure may change. The greatest change occurs at the junctions and bifurcations. In this regard, we decided to study the renal artery bifurcation just where the renal arteries divide into smaller arteries before entering the kidney.

**Methods:** The structure of renal artery bifurcation was assessed in six normal male dogs by light microscopy. Also the thickness of the tunica intima, tunica media, and tunica adventitia before and after bifurcation area was measured.

**Results:** Tunica intima cannot be seen in this area and tunica media in one side and tunica adventitia in other side were very thick. It seems that division of renal artery happens with the penetration of tunica adventitia in one side and tunica media in the other side.

**Conclusion:** In this area, the artery has thick tunica media on one side and thick tunica adventitia on the other side and these differences were significant. The circular and longitudinal smooth muscle cells can be seen in renal artery bifurcation. These structures may be due to function of this area to maintain and control blood pressure and prevent artery from bursting and dilation.

### **Key Words:**

Renal artery, Smooth muscle cell, Elastic fibers, Dog

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## 1. Introduction

**T**he circulatory system consists of the heart and blood vessels that transport blood throughout the body. Blood vessels can be divided into three categories: arteries, which carry blood from the heart to all organs and muscles of the body; capillaries which are extremely small vessels located within the body tissues where the actual exchange of water and chemical compounds take place between the blood and the tissues; and finally veins, which return blood from the capillaries to the heart. Microscopic anatomy of arteries shows three layers in the blood vessels walls. The inner most layer is tunica intima consisting of endothelium and subendothelial connective tissue. Then tunica media which consists of smooth muscle cell layers in the circular form, collagen and elastic fibers in varying amounts, elastic lamellae, and proteoglycans. Smooth muscle cells are responsible for making proteoglycans. Collagen fibers are the manufacturer of tunica adventitia. Vascular smooth muscle cells, proteins, collagen fibers and elastin as the extracellular matrix play a central role in the biomechanics of vascular tissue [1].

The arteries are the large blood vessels with higher blood pressure than other parts of the circulatory system. This high pressure can affect the ability of the arteries to open and close [2]. Structural variation of the arteries has always attracted researchers. Studies on the arteries have shown dramatic changes at the site of conversion from elastic artery to muscular artery, i.e., it has been shown that the structure of renal artery in humans and rats at its origin from abdominal aorta is different from abdominal aorta and renal artery structure [3, 4, 5]. This different structure has been observed in human carotid artery [6] and the branches of the abdominal aorta in sheep and lamb, too [7]. In a study of renal artery in dogs, it has been shown that an area in the proximal part of the renal artery is different from abdominal aorta and renal artery [8]. The continuation of elastin from the aorta into its branches [9] and noticeable changes in the pattern of elastin at aorta branches on the proximal and distal lips of the junction has been already discussed [10]. Despite numerous studies on the structure of arteries in the branching of aorta, there is no research on the division of the arteries. Each renal artery divides into two branches at or very close to the hilum of the kidney [11]. Therefore, we decided to investigate the structure of renal artery in bifurcation area. This area can play an important role in regulating blood pressure because blood pressure in arteries varies during the cardiac cycle. It is maximum when the heart contracts and minimum when heart re-

laxes and also varies in different arteries and decreases with more distance from the heart.

## 2. Materials and Methods

### Animals

Six renal arteries were harvested from three clinically healthy male dogs after they were euthanized with an overdose of thiopental sodium. Then their abdominal cavities were opened and the renal arteries exposed.

### Histological preparation

For histology, 6 left and right renal arteries were freed from their aortic origin to the renal hilus and all samples were taken from renal artery bifurcation just where the renal arteries are divided into smaller arteries before entering to the kidney. They were taken from dogs that were used within the Discipline of Surgical Technique of the School of Veterinary of Kazeroon in regular classes within the medical course. These animals were killed in accordance with the technical standards of the Regional Council of Veterinary Medicine and the Society for Animal Protection.

The specimens were immersed in 10% formalin solution for 48 h. After dehydration through a graded series of alcohol solutions, the samples were cleared in Xylene and embedded in paraffin. The paraffin blocked tissue was cut into 6- $\mu$ m serial sections. Each fifth section was mounted and stained with orcein, and the bifurcation of the renal artery investigated by light microscope. Thickness of the tunica intima, tunica media, and tunica adventitia before and after renal artery bifurcation was measured by transferring the image from the microscope to the computer screen, by Dino Kilcher software.

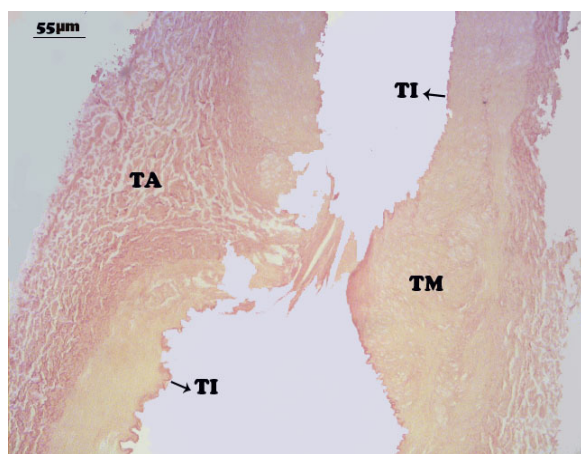
### Statistical analysis

All obtained data are presented as mean $\pm$ S.E.M. Means of groups were compared by 1-way analysis of variance (ANOVA) then paired t test analysis was performed to assess comparisons. The level of statistical significance was accepted as  $\leq 0.05$ . Calculations were performed using the SPSS version 13.0 (SPSS Inc., Chicago, Ill, USA).

## 3. Results

### Microscopic observations

Structure of renal artery bifurcation before entering the kidney is shown in Figures 1 to 4. As it can be seen in the images, there are significant changes in renal artery



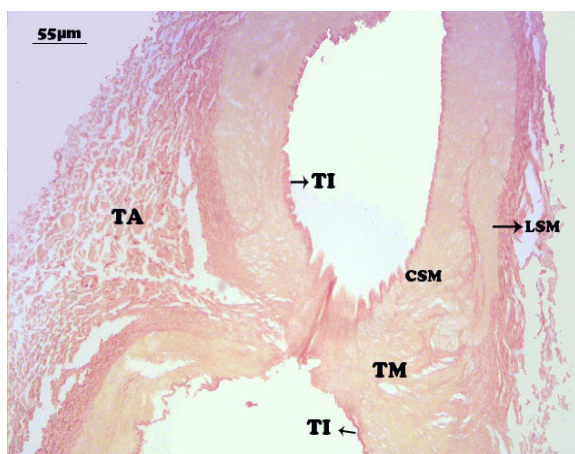
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**Figure 1.** Microscopic images of the renal artery bifurcation in male dogs

Tunica Intima (TI), Tunica Media (TM), Tunica Adventitia (TA), Orcein,  $\times 175$ . Bar 55  $\mu\text{m}$

bifurcation. Tunica intima cannot be seen in this area and tunica media in one side and tunica adventitia in other side comprised the wall thickness. It seems that renal artery bifurcates with penetration of tunica adventitia in one side, and tunica media in the other side (Figure 1). In the bifurcation zone, the thickness of tunica media ( $20.45 \pm 5.95$ ) and tunica adventitia ( $25.82 \pm 6.56$ ) were significantly higher compared with before bifurcation (tunica media:  $12.80 \pm 4.62$ , tunica adventitia:  $7.62 \pm 3.13$ ) and after it (tunica media:  $9.92 \pm 6.29$ , tunica adventitia:  $4.39 \pm 0.63$ ) ( $P \leq 0.05$ ) (Figure 5 and Table 1).

Along with more penetration of tunica adventitia in one side and tunica media in the other side of the artery, division of the renal artery gradually happens. Tunica media of renal artery in division area has two orientation of smooth muscle cell, circular and longitudinal form (Figure 2). With the appearance of the tunica media on both sides of renal artery bifurcation, renal artery is converted into two connected branches. In both branches, tunica intima reach to other side and form a continuous layer. But tunica adventitia is thicker on one side compared with



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**Figure 2.** Microscopic images of the renal artery bifurcation in male dogs

Tunica Intima (TI), Tunica Media (TM), Tunica Adventitia (TA), Circular Smooth Muscle Cells (CSMC), Longitudinal Smooth Muscle Cell (LSMC), Orcein,  $\times 175$ . Bar 55  $\mu\text{m}$

the other side (Figure 3). Finally, renal artery is divided into two smaller arteries and each artery has tunica intima, tunica media, and adventitia (Figure 4).

#### 4. Discussion

Owing to renal artery bifurcation before getting in to the kidney artery show considerable different in structure. The structure of the artery enables it to perform its function more efficiently. The walls of arteries are made up of three layers. Tunica intima is composed of endothelial cells and minimize the blood flow friction. The tunica media contains smooth muscles, collagen, and large amount of elastic fibers.

The main cells of the vascular wall are smooth muscle cells. By producing the extracellular matrix during development, smooth muscle cells provide the capacity of withstanding the high pressure and arterial repair after injury in adult life. Vascular smooth muscle cells of resistance vessels participate in the regulation of blood

**Table 1.** Comparison of Mean $\pm$ SD of tunica intima, tunica media and tunica adventitia thickness of renal artery before, after, and at the bifurcation zone

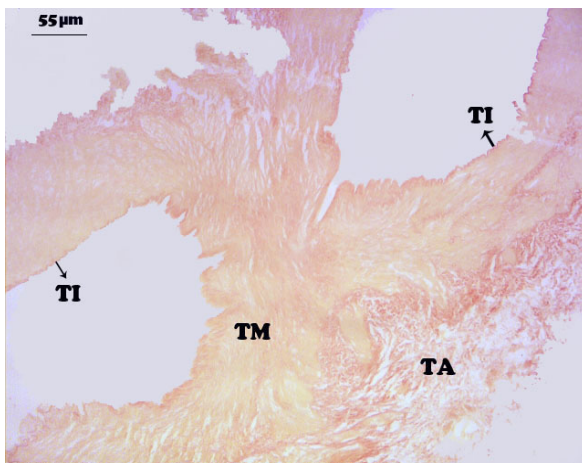
	Tunica Intima	Tunica Media	Tunica Adventitia
Bifurcation zone	-	$20.45 \pm 5.95$	$25.82 \pm 6.56$
Before bifurcation	$0.55 \pm 0.02$	$12.80 \pm 4.62^*$	$7.62 \pm 3.13^*$
After bifurcation	$0.78 \pm 0.05$	$9.92 \pm 6.29^*$	$4.39 \pm 0.63^*$

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Values are expressed as Mean $\pm$ SD. By using 1-way ANOVA the results are significant at  $P < 0.05$ .

\*: Significant difference compared to bifurcation zone

•: Significant difference compared to bifurcation zone



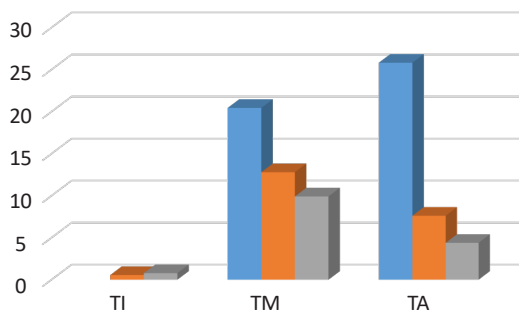
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**Figure 3.** Microscopic images of the renal artery bifurcation in male dogs

Tunica Intima (TI), Tunica Media (TM), Tunica Adventitia (TA), Orcein,  $\times 175$ . Bar 55  $\mu\text{m}$

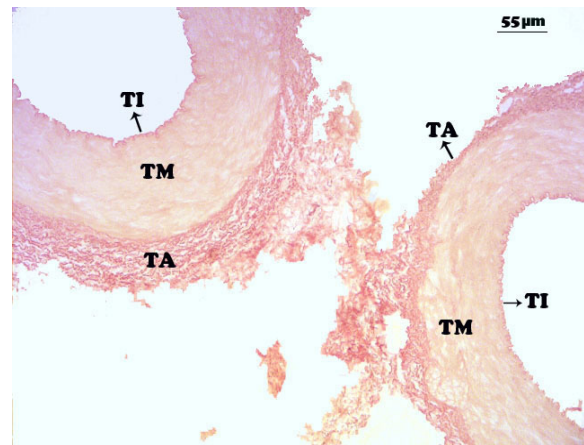
pressure, too [12]. Smooth muscle cells can control the volume of blood flowing into a tissue at different times by contracting and narrowing the diameter of the arteries [2]. Vascular wall contraction and regulation of the size of the blood vessel is the other function of smooth muscle cells [13]. One study reported that smooth muscle cells in anterior aorta can help the aorta to supply blood for the head, neck, and upper limbs [14].

Elastic fibers are responsible for the elasticity of blood vessels. The elastic fibers in the blood vessels basically allow them to expand and return to their original size. These changes in size and diameter happens in arteries as blood rushes out of the heart in high pressure which stretches and widens walls of arteries. However, when the blood gets out of the arteries they return to their original size. It has been reported that elastin is an important elastic component that provides extensible tissues with resilience [15]. In the walls of the arteries, especially in



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**Figure 5.** Comparison of tunica intima, tunica media, and tunica adventitia of renal artery thickness before, after and at bifurcation zone



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**Figure 4.** Microscopic images of the renal artery bifurcation in male dogs

Tunica Intima (TI), Tunica Media (TM), Tunica Adventitia (TA), Orcein,  $\times 175$ . Bar 55  $\mu\text{m}$

large ones, elastic rebound helps maintain arterial pressure [16]. The main components of elastic fibers, i.e., elastin- and fibrillin-containing microfibrils play structural and mechanical role in the arteries and provide elasticity and resilience to the tissues [17]. The elasticity of the walls is important as it reduces the possibility of their rupture. In rat neonates, pulling aorta will rupture it because its elastic layer structure is incomplete and the tensile strength is insufficient [18].

Tunica adventitia is made of elastic and large amount of collagen fibers. Collagen is the main protein of the connective tissue and represents about one-fourth of the total protein content in many animals [19]. Collagen fibers have important role in blood vessels. The walls of the veins, arteries, and capillaries are made of collagen. Collagen fibers give the vessels their strength, structure and flexibility. The collagenous fibers give tensile strength to the blood vessels walls. In other words, it is collagenous fibers that prevent the blood vessels rupture or get overly dilated when blood pressure is high. The high relative collagen content of the adventitia helps prevent vascular rupture at extremely high pressures [20].

Although the three mentioned layers can be seen in all arteries but in some areas, the overall structure of the artery show changes. These changes can be seen when the artery branches or bifurcates [5]. It has been reported that in renal artery by reducing the size of the vessel, the elastic lamellae in the media becomes thinner so that they disappear completely in the distal renal artery. The proximal end of the renal artery and the common carotid artery in humans and rats have different structures compared to other parts of these arteries. A segment has been seen



in the proximal part of these arteries in which the elastic type wall architecture is replaced by one of the muscular type [21]. Studies of the vertebral artery in the giraffe have shown that the caudal segment of the vertebral artery has an elastic structure, while the cranial segment has a muscular structure. The transition of the arterial wall normally occurs between C7 and C5. It involves diminution of the elastic tissue in the luminal portion of the tunica media and a simultaneous increase of the smooth muscle cells content [22]. Examination of the musculophrenic and superior epigastric arteries has shown that the media of the first 1 to 2 cm of these arteries is elastomuscular or muscular with few elastic lamellae, whereas more distally the media is purely muscular [23].

Results of the present study clearly show that renal artery structure change significantly compared to other parts at the bifurcation. In this area, the tunica media on one side and the tunica adventitia on the other side form the thickest layer. In the bifurcation zone, the thickness of tunica media and tunica adventitia is significantly higher compared with before and after bifurcation ( $P \leq 0.05$ ). Similarly, tunica media and tunica adventitia of the renal artery bifurcation has more thickness compared with other parts of the artery. Tunica adventitia in arteries is a collagen-rich connective tissue containing fibroblasts and perivascular nerves [24] while tunica media is made up a large number of smooth muscle cells and elastic fibers [25]. Thus by increasing the thickness of the tunica media and tunica adventitia and their constituent compounds, the smooth muscle cells in tunica media and collagen fibers in tunica adventitia will increase in renal artery bifurcation just where the renal artery divides into smaller arteries and enter the kidney. When the blood reaches the renal artery bifurcation it divides and flows into two smaller arteries so the blood pressure decreases but there is an accumulation of blood in the renal artery bifurcation. In this area, the volume of blood flow into the smaller arteries and the blood pressure should be controlled to prevent bursting the arteries. In large arteries like abdominal aorta, arterial pressure is maintained by elastic fibers but in muscular arteries such as renal artery, the pressure remains high by contraction and relaxation of smooth muscle cells [2]. Thus, smooth muscle cells maintain and control blood pressure, while collagen fibers prevent arteries from bursting and dilation.

In this study, we observed two orientation of smooth muscle cells in renal artery bifurcation; circular form and longitudinal form. However, the fact that the orientations of smooth muscle cells in this area are similar to the muscular veins is of special interest. The blood vessels are dilatable when elastic and collagen fibers are more than

smooth muscle cells in their walls and they are contractile if smooth muscle cells are more in their wall. The contractile state of the vein wall plays an important role in blood flow [26].

In this study, we proved that structure of renal artery bifurcation is different with other parts of the renal artery. In this area, the artery has thick tunica media on one side and thick tunica adventitia on the other side compared with other parts of the artery. The circular and longitudinal smooth muscle cells can be seen in renal artery bifurcation. These changes where the renal artery divides into two branches may be due to the function of this area to maintain and control blood pressure to prevent artery from dilation and bursting.

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### Conflict of Interest

The authors declared no conflicts of interest.

### References

- [1] Saez P, Garcia, Pena E, Gasser TC, Martinez MA. Microstructural quantification of collagen fiber orientations and its integration in constitutive modeling of the porcine carotid artery. *Acta Biomaterialia*. 2016; 33:183-193. doi: [10.1016/j.actbio.2016.01.030](https://doi.org/10.1016/j.actbio.2016.01.030)
- [2] Junqueira LC, Carneiro J, Kelly RO. *Basic histology (text and atlas)*. 11<sup>th</sup> edition. New York: McGraw-Hill; 2005.
- [3] Baardwijk C, Barwick SE, Roach MR. Organization of medial elastin at aortic junction in sheep and lambs. *Canadian Journal of Physiology and Pharmacology*. 1985; 63(7):855-862. doi: [10.1139/y85-140](https://doi.org/10.1139/y85-140) [10.1139/y85-140](https://doi.org/10.1139/y85-140)
- [4] Janzen J, Lanzer P, Rothenberger-Janzen K, Vuong PN. The transitional zone in the tunica media of renal arteries has a maximal length of 10 millimeters. *Vasa*. 2000; 29(3):168-172. doi: [10.1024/0301-1526.29.3.168](https://doi.org/10.1024/0301-1526.29.3.168)
- [5] Osborn-Pellegrin M. Some ultrastructural of the renal artery and abdominal aorta in the rat. *Journal of Anatomy*. 1978; 25(pt 3): 641-652. PMID: [PMCID: PMCID:1235630](https://pubmed.ncbi.nlm.nih.gov/1235630/)
- [6] Janzen J, Lanzer P, Rothenberger-Janzen K, Vuong PN. Variable extension of the transitional zone in the medial structure of carotid artery tripod. *Vasa*. 2001; 30(2):101-106. doi: [10.1024/0301-1526.30.2.101](https://doi.org/10.1024/0301-1526.30.2.101)

- [7] Roach MR. The structure and elastic properties of arterial junction. *Connective Tissue Research*. 1987; 15(1-2):77-84. doi: 10.3109/03008208609001976
- [8] Ramezani Nowrozani F. Structure of the orifice of the renal artery in the abdominal aorta in adult male dog. *Iranian Journal of Veterinary Research*. 2011; 12(1):67-72. doi: 10.22099/ijvr.2011.44
- [9] Van Baardwijk C, Barwick SE, Roach MR. Organization of medial elastin at aortic junction in sheep and lambs. *Canadian Journal of Physiology and Pharmacology*. 1985; 63(7):855-862. doi: 10.1139/y85-140
- [10] Rees PM. Electron microscopical observations on the architecture of the carotid arterial walls. With special reference to the sinus portion. *Journal of Anatomy*. 1968; 103(pt 1): 33-47. PMID: PMC1231873
- [11] Yadav A, Yadav M, Dixit A. A research on the Incidence of renal vessel arrangement at hilum of kidney. *Scholars Journal of Applied Medical Sciences*. 2014; 2(5C):1715-1716.
- [12] Lacolley P, Regnault V, Nicoletti A, Li Z, Michel JB. The vascular smooth muscle cell in arterial pathology: A cell that can take on multiple roles. *Cardiovascular Research*. 2012; 95(2):194-204. doi: 10.1093/cvr/cvs135.
- [13] Brozovich FV, Nicholson CG, Degen CV, Gao YZ, Aggarwal M, Morgan KG. Mechanisms of Vascular Smooth Muscle Contraction and the Basis for Pharmacologic Treatment of Smooth Muscle Disorders. *Pharmacological Reviews*. 2016; 68(2):476-532. doi: 10.1124/pr.115.010652.
- [14] Shakuntala Rao N, Sujatha K, Meera K, Krishna Rao HR. A comparative study on the structure and function of aorta in man and ruminant animals. *International Journal of Anatomy and Research*. 2016; 4(4):3194-98. doi: http://dx.doi.org/10.16965/ijar.2016.437
- [15] Faury G. Role of elastin in the development of the vascular function. Knock-out study of the elastin gene in mice. *Journal of the Society of Biology*. 2001; 195(2):151-156. doi: 10.1051/jbio/2001195020151
- [16] Ushiki T. Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from a morphological viewpoint. *Archives of Histology and Cytology*. 2002; 65(2):109-126. doi: 10.1679/aohc.65.109
- [17] Lannoy M, Slove S, Jacob MP. The function of elastic fibers in the arteries: Beyond elasticity. *Pathologie Biologie*. 2014; 62(2):79-83. doi: 10.1016/j.patbio.2014.02.011.
- [18] Berillis P. The Role of Collagen in the Aorta's Structure. *The Open Circulation and Vascular Journal*. 2013; 6(1):1-8.
- [19] Utako Yokoyama U, Tonooka Y, Koretake R, Akimoto T, Gonda Y, Saito J, et al. Arterial graft with elastic layer structure grown from cells. *Scientific Reports* 7. 2017; 140(2017). doi: 10.1038/s41598-017-00237-1.
- [20] Burton AC. Relation of structure to function of the tissues of the wall of blood vessels. *Physiological Reviews*. 1954; 34(4):619-642. doi: 10.1152/physrev.1954.34.4.619
- [21] Janzen J. The microscopic transitional zone between elastic and muscular arteries. *Archives Des Maladies Du Coeur Et Des Vaisseaux*. 2004; 97(9):909-914. PMID: 15521485
- [22] Kimani JK. Structural organization of the vertebral artery in the giraffe (*Giraffa camelopardalis*). *The Anatomical Record*. 1986; 217(3):256-262. doi: 10.1002/ar.1092170306
- [23] Van Son JA, Smedts FT, Wilde PC, Pijls NH, Wong-Alcala L, Kubat K, et al. Histological study of the internal mammary artery with emphasis on its suitability as a coronary artery bypass graft. *The Annals of Thoracic Surgery*. 1993; 55(1):106-113. doi: 10.1016/0003-4975(93)90483-x
- [24] Majesky MV, Dong XR, Hoglund V, William M, Mahoney Jr, Daum G. The adventitia: a progenitor cell niche for the vessel wall. *Cells Tissues Organs*. 2012; 195(1-2):73-81. doi: 10.1159/000331413.
- [25] Wagenseil J, Mecham, RP. Vascular Extracellular Matrix and Arterial Mechanics. *Physiological Reviews*. 2009; 89(3):957-989. doi: 10.1152/physrev.00041.2008.
- [26] Fourman JD, Moffat D. *The blood vessels of the kidney*. Edinburgh: Blackwell; 1971.